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# Epidemiological Dynamics of *Xanthomonas xonopodis* pv. *phaseoli* in French bean (*Phaseolus vulgaris*): Unraveling the Factors Governing Bacterial Blight Pathogenesis

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## ABSTRACT

The study was conducted during May, 2020 to June, 2021 in the Department of Plant Pathology Laboratory of Dr. Y.S Parmar University of Horticulture and Forestry, Nauni, located in Solan district of Himachal Pradesh, India to study the role of various epidemiological parameters on the development of common bacterial blight of French bean. The diseased samples were collected and the pathogen was isolated and purified on Luria Bertani (LB) agar plates. The pathogenicity test was performed on the potted plants as well as on the detached leaves using carborundum abrasion and pin prick method of inoculation. The incubation period on potted plants using carborundum abrasion and pin prick method revealed the initial symptoms after 117.60 h and 120.30 h, respectively, while on detached leaves they were visible after 72.30 h and 84.50 h of inoculation, respectively. Further to study the effect of different epidemiological factors on disease development, the leaves inoculated using carborundum abrasion were subjected to four temperature levels, five relative humidity levels and five different durations of leaf wetness under controlled conditions in relative humidity cum temperature control cabinet. The observations were recorded at different intervals and the data revealed that the pathogen caused severe symptoms on the leaves with high apparent rate of infection under 30°C temperature, 100% RH and a maximum of 24 h of leaf wetness. It was observed that the severity of disease decreased as the temperature raised from 30°C and increased with the rise in RH levels and leaf wetness duration.

**KEYWORDS:** Bacteria, bean, disease, durations, temperature, symptoms

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***Data Availability Statement:*** Legal restrictions are imposed on the public sharing of raw data. However, authors have full right to transfer or share the data in raw form upon request subject to either meeting the conditions of the original consents and the original research study. Further, access of data needs to meet whether the user complies with the ethical and legal obligations as data controllers to allow for secondary use of the data outside of the original study.

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## 1. INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is a highly significant legume crop that holds great importance in the plant family Fabaceae/Leguminosae. It is cultivated in various climatic zones, including tropical, subtropical, and temperate regions across the globe (Prakash and Ram 2014; Adila et al., 2021).In India, it is grown over an area of 0.21 million hectare with a production of 2.08 million tonnes (Anonymous, 2022a). In Himachal Pradesh it covers an area of about 3893 ha with a production of 49916 metric tonnes (Anonymous, 2022b).The major bean growing districts of the state are Shimla, Solan, Sirmour, Chamba, Kinnaur, Kullu and some areas of Mandi. The cropis consumed as vegetable when the pods are immature, delicate and tender. It is rich in protein and other micronutrients like calcium, folate iron, zinc, magnesium, phosphorus, potassium and vitamin B (Beebe et al., 2014).It is rich in thiamine, riboflavin, niacin and biotin besides containing phytochemicals like polyphenolic compounds which prevents various disorders like cardiovascular diseases, colon cancer, obesity and diabetes (Petry et al., 2015). Due to the presence of these qualities, it is considered as a 'grain of hope' in various under-developed and developing countries. It is also known as the 'meat of the poor' due to the presence of essential proteins (Kanwar and Mehta, 2018). The successful and cost-effective cultivation of beans is influenced by the presence of different diseases caused by fungal, bacterial, and viral pathogens. Among the various diseases affecting beans, common bacterial blight (CBB) caused by *Xanthomonas axonopodis* pv. *phaseoli* is a highly destructive disease resulting in significant yield reductions globally (Osdaghi et al., 2009; Girma et al., 2022a).The pathogenis a non-spore forming, rod shaped, gram negative aerobic bacterium having a single polar flagellum (Belete et al., 2017). The extent of yield losses caused by the disease can fluctuate between 10% and 40%, with variations determined by factors such as the susceptibility of different bean cultivars and the prevailing environmental conditions (Saettler, 1989). The disease manifests as a foliar disease with symptoms such as water-soaked lesions on leaves, wilting, necrosis, and the characteristic "bird's eye" spots on pods. Symptoms on the seeds appear as butter yellow spots that turn into brown spots (Chen et al., 2021). Severely infected seed may be shrivelled, affecting their germination rate and vigour (Darrasse et al., 2018; Aziziaram et al., 2021). In addition to the visual damage, CBB-infected beans often suffer from reduced market quality and nutritional value, making it a major concern for both farmers and consumers (Akhavan et al., 2013). Common bacterial blight primarily thrives in warm climatic conditions and higher humidity levels. It exhibits its most destructive effects when temperatures range between 28°C and 32°C and relative humidity is more than 85% (Emam et al., 2010). Asthe temperatures and relative humidity increases, favorable conditions are created for the outbreak and spread of common bacterial blight (CBB) epidemics in susceptible common bean varieties (Torres et al., 2009; Girma et al., 2022b). Understanding the role of epidemiological factors like temperature, relative humidity and leaf wetness duration in the development and spread of this disease is crucial for itseffective management strategies. So, the primary objective of this research paper was to explore how different environmental factors influence the development and severity of bacterial blight in common bean.

## 2. MATERIALS AND METHODS

### *2.1. Isolation of the pathogen*

The study was conducted in the Plant Pathology Laboratory, Dr Y.S Parmar University of Horticulture and Forestry, Nauni, in Solan district of Himachal Pradesh, India during May, 2020 to June, 2021. The university is located at a geographical position of 30.8653° North latitude and 77.1698° East longitude of the globe. The diseased plant samples collected from different bean growing areas of Solan and Sirmaur districts of Himachal Pradesh were subjected to microscopic examination and various biochemical tests to confirm the presence of the associated bacterium. The samples having young characteristic 'V' shaped lesions and showing the presence of ooze were selected for isolation of the pathogen. The diseased leaves were washed with sterile water and small bits were cut from the edges of the lesions containing some healthy portion. The bits were surface sterilized using sodium hypochlorite (5%) solution followed by series of washing with sterilized distilled water. The surface sterilized bits were then transferred to a flask containing sterilized distilled water and kept on a mechanical shaker for at least 2 hours a day for 2–3 days. A loopful of the bacterial suspension was streaked on nutrient agar plates and further incubated at 28±1°C for 48 h. The plates were examined for the development of colonies and purified and multiplied on LB (Luria - Bertani) media (Sultana et al., 2018).

### *2.2. Identification of the pathogen*

The test bacterium was identified on the basis of various morphological, cultural and biochemical characters as described in "Experiments in Microbiology Plant Pathology Tissue Culture and Microbial Biotechnology" by Aneja (2018). To study the shape and size of the bacterium, Dorner's nigrosin method of negative staining was used. The solution was prepared by dissolving nigrosin (7.0 g) in distilled water (100 ml) in a conical flask. The flask was immersed in a boiling water bath for 30 minutes and after that formalin (0.5 ml) was added as a preservative.

The solution was filtered twice through double filter paper and the filtrate was further used as a stain. A loopful of two days old bacterial suspension was mixed with the staining solution on a glass slide and spread into a thin film, which was further air dried and examined under the microscope. The size of the bacterial cells was measured by the ocular micrometer and shape was observed with dark grey background under the microscope. To study the gram-positive or gram-negative behaviour of the bacterium Gram's staining was done. Further various biochemical tests including oxidase test, catalase test, nitrate reductase, starch hydrolysis, gelatine hydrolysis, hydrogen sulphide production and potassium hydroxide test, were performed for the confirmation of the bacterium.

*2.3. Pathogenicity*

*2.3.1. Raising of plants*

Seeds of bean cultivar Contender were sown in plastic pots (9 cm dia) filled in with sterilized soil. The pots were then kept in greenhouse and irrigated at regular intervals. Three week old plants were used for inoculation.

*2.1.2. Inoculum preparation*

For inoculation, bacterial cell suspension was prepared from 48 hr old purified culture of the test pathogen raised on LB plates. The bacterial colonies were dissolved in 100ml sterilized nutrient broth and put on a mechanical shaker for 24–36 hours. The colonies were further diluted by using serial dilution method (upto seven dilutions) to obtain a concentration of $1\times10^7$cfu $ml^{-1}$.

*2.1.3. Inoculation method*

The prepared inoculums was then inoculated on three week old potted plants as well as on detached leaves using two different methods viz; carborundum abrasion and pin prick method. The leaf surface was slightly rubbed with the forefinger or cheese cloth using carborundum powder in a direction opposite to the growth of leaf hairs to cause injury and the inoculation was done by rubbing the cotton swab dipped in bacterial suspension ($10^7$cfu $ml^{-1}$) on the entire leaf. Whereas, in pin prick method the moist leaves were pin pricked on the lower surface along the veins and inoculated with the bacterial suspension ($10^7$cfu $ml^{-1}$) using a micropipette. In both the methods the plants and leaves inoculated with sterile distilled water were kept as control. The inoculated plants and the detached leaves kept in Petriplates covered with moist filter paper wereinoculated in relative humidity cum temperature control cabinet adjusted at 28°±2°C and RH of 90%. Observations were made for the development of symptoms at regular intervals and after the appearance of symptoms, the bacterium was re-isolated and compared with the original isolate to prove the Koch's postulates.

*2.3. Effect of temperature, relative humidity and leaf wetness duration on the progression of disease*

The inoculated leaves were kept in Petriplates covered on both sides by moist filter paper and then incubated at different temperatures of 20, 25, 30 and 35°C and different relative humidity levels of 75, 85, 90, 95 and 100% in relative humidity cum temperature control cabinet in separate sets. Similarly different sets of inoculated leaves were kept in the cabinet to check the effect of leaf wetness duration on disease progression. The leaves were removed after interval of 2, 4, 8, 16 and 24 h from the relative humidity cum temperature control cabinet and dried under the fan before transferring them again to the cabinet.Ten leaves were taken per replication for each epidemiological factor and each treatment was replicated five times. The incubation period was recorded and the leaf area infected after 2, 4, 6 and 8 days of the incubation period was recorded to compute the % disease severity and apparent rate of infection. The % disease severity was recorded according to the scale given by Dursun et al. (2002) (Table 1).

% disease index was calculated by using the following formula (Mckinney, 1923) as given below:

$$\%\ \text{Disease index}=(\sum (ni\times vi))/V\times N\times 100 \quad \ldots\ldots\ldots\ldots(1)$$

ni=number of leaves with the respective disease rating;

vi=Disease rating
V=The highest disease rating
N=The number of leaves observed

The disease progression at different temperature, RH and leaf wetness levels was measured by calculating the apparent infection rate (r) as per Vander Plank (1963) using logistic equation as given below:

$$r=(2.302/t_2-t_1)\log10\{X_2(1-X_1)/X_1(1-X_2)\} \quad \ldots\ldots\ldots\ldots(2)$$

r=Apparent rate of infection per day

$t_2 - t_1$=Time interval between first and last observation

$X_1$ & $X_2$=Proportion of leaf area covered by the lesion at $t_1$ and $t_2$ time intervals, respectively

$(1-X_1)$ and $(1-X_2)$=Proportion of healthy leaf area at $t_1$and $t_2$ time intervals, Respectively

Table 1: Disease rating scale for scoring the % disease severity

| Disease rating | Leaf area infected |
|---|---|
| 0 | No disease |
| 1 | 0.1–10% leaf area infected |
| 2 | 10.1–25% leaf area infected |
| 3 | 25.1–50% leaf area infected |
| 4 | 50.1–75% leaf area infected |
| 5 | >75% leaf area infected |

### 2.5. Statistical analysis

Analysis of variance for experiments conducted under controlled conditions and laid out as per CRD was done as per the methodology suggested by Gomez and Gomez (1984). The replication and treatment mean sum of square was tested against error mean squares by 'F' test at (t-1), (t) (r-1) degree of freedom for CRD at 0.05 level of significance. The calculated F-values were compared with tabulated F-value. Wherever F-test was found significant, the critical difference was calculated to find out the superiority of one treatment over the others.

## 3. RESULTS AND DISCUSSION

### 3.1. Isolation of the pathogen

Growth of the isolated bacterium obtained on nutrient agar medium after 48 hours of incubation was observed as pale-yellow coloured, smooth and mucoid colonies. The test bacterium was further purified on Luria-Bertani (LB) agar medium. The colonies obtained were smooth, shiny and dark yellow coloured after 48 hours of incubation at 28±2°C. The results are in agreement with the findings of Schaad (1988) who observed yellow coloured, mucoid, glistering and convex colonies with entire margins on nutrient agar media. AlsoSultana et al. (2018) observed straw yellow coloured and glistening colonies on Luria-Bertani (LB) agar media.

### 3.2. Identification of the pathogen

The shape of the isolated bacteria was observed as straight rods with rounded edges under the microscope. The average size recorded was 0.52×1.36 $\mu m^2$. The bacterium retained the colour of safranin i.e. pink colour, therefore confirmed to be gram negative. The results of various biochemical tests were positive for carbohydrate utilization, starch hydrolysis, gelatin liquefaction, hydrogen sulphide production, catalase test and potassium hydroxide test whereas the results were negative for nitrate reduction, and oxidase test (Table 2). The methodology used was similar to that of Bergey et al. (1939); Bradbury, 1984 and Lelliot and Stead, 1988. (The references used here are the books and manuals used for the methodology)

Table 2: Reaction of bacterium to different biochemical tests

| Sl. No. | Tests | Reactions | Observations |
|---|---|---|---|
| 1. | Gram's staining | Negative | Retained the colour of safranine |
| 2. | Carbohydrate utilization | Positive | Yellow colour development |
| 3. | Starch hydrolysis | Positive | Development of clear zone around bacterial growth |
| 4. | Nitrate reduction | Negative | No colour change |
| 5. | Gelatin liquefaction | Positive | No solidification of the media |
| 6. | Hydrogen sulphide production | Positive | Formation of black precipitates |
| 7. | Oxidase test | Negative | No colour change |
| 8. | Catalase test | Positive | Gas bubble formation |
| 9. | Potassium hydroxide test | Positive | Formation of a slimy streak |

### 3.3. Pathogenicity

Irrespective of the inoculation techniques used for pathogenicity test, initial symptoms appeared much earlier on detached leaves as compared to the potted plants. The study further revealed that the bacterial infection in both the cases (potted plants and detached leaves) was much rapid through abrasion method by the pre-inoculation application of carborundum powder than the infection caused by pin prick method. The development of the yellow fleck as initial symptom was observed after 117.60 h and 120.30 h of inoculation, respectively in potted plants, while on detached leaves initial symptoms were visible after 72.30 h and 84.50 h of inoculation, respectively. Further the symptom development also varied in both the cases (Table 3). The bacterium was re-isolated and then inoculated into the healthy plants. It was observed that similar symptoms were recorded on the inoculated healthy plants, hence proving the Koch's postulates. Similarly, Tolba et al. (2017) while performing pathogenicity tests for *Xanthomonas citri* pv. *citri* using carborundum abrasion method of inoculation on detached leaf as well as plants of citrus observed the initial symptoms on the detached leaves after 5–7 days of inoculation whereas the symptoms were observed after 8–10 days on the citrus plants. Ebrahim (2014) also conducted pathogenicity experiment for *Xanthomonas axonopodis* on mungbean. Two-day old bacterial inoculum ($1\times10^7$cfu $ml^{-1}$) was spray inoculated on fully expanded leaves of 20 day old mungbean plants and the symptoms were observed after 8–10 days after inoculation.

### 3.3. Effect of temperature, relative humidity and leaf wetness duration on the progression of disease

#### 3.3.1. Temperature

It was observed that the temperature of 30°C was optimum for the development of initial symptoms of bacterial blight in a minimum incubation period of 84.4 h after inoculation. Thedisease progression was recorded in significantly high proportion (84.8%) at 30°C with higher apparent rate of infection (0.33unit $day^{-1}$) after 8 days of incubation period

Table 3: Pathogenicity of *Xanthomonas axonopodis* pv. *phaseoli*

| Method of inoculation | Incubation period (h) | |
|---|---|---|
| Carborundum abrasion | Potted plants | 117.6 |
| | Detached leaf | 72.3 |
| Pin prick method | Potted plants | 120.3 |
| | Detached leaf | 84.5 |
| Symptom development | Potted plants | The initial symptoms on the leaves were observed as small, watersoaked spots which were surrounded by yellow halo. The spots further increase in size and become dark brown in colour and coalesced together manifesting blighted appearance on the leaves. |
| | Detached leaf | Initially a small yellow fleck with small light brown centre appeared on the leaves which further enlarged and formed circular necrotic lesions surrounded by yellow halo. With the progression of disease the increase in size of lesions and the coalescing of these lesions resulted in blighted appearance on the inoculated leaves. |

followed by 25°C and 35°C with apparent rate of infection of 0.30unit day$^{-1}$ and 0.28unit day$^{-1}$, respectively. Whereas, the pathogen failed to produce any type of symptoms at 20°C (Table 4). The results were similar to the findings of Shukla and Gupta (2005), as they indicated the rapid disease progression of bacterial spot of tomato at 25°C and 30°C. Simultaneously, Hallu et al. (2017) while observing effect of temperature on the development of bacterial blight of bean recorded significantly high disease progression between 25°C-30°C whereas moderate disease development was observed at 35°C.

Table 4: Bacterial blight progression at different temperature regimes

| Temperature (°C) | Incubation period (h) | % disease severity after days of incubation period | | | | Mean | Apparent infection rate (r) (unit day$^{-1}$) |
|---|---|---|---|---|---|---|---|
| | | 2 | 4 | 6 | 8 | | |
| 20 | - | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | - |
| 25 | 89.6 | 11.20 (19.38) | 35.20 (36.32) | 56.00 (48.45) | 73.60 (59.16) | 54.93 (47.98) | 0.30 |
| 30 | 84.4 | 14.40 (22.07) | 50.40 (45.21) | 68.40 (55.85) | 84.80 (67.33) | 67.86 (56.15) | 0.33 |
| 35 | 98.6 | 8.80 (16.98) | 29.60 (32.90) | 45.20 (42.22) | 62.80 (52.45) | 45.86 (42.52) | 0.28 |
| Mean | | 8.60 (14.61) | 28.80 (28.60) | 42.40 (36.65) | 55.30 (44.75) | | |
| SEm± | 1.52 | Temperature=0.71, Interval = 0.71<br>Temperature×Interval = 1.41 | | | | | |
| CD ($p$=0.05) | 4.61 | Temperature=2.01, Interval=2.01<br>Temperature×Interval=4.01 | | | | | |

*3.3.2. Relative humidity*

A delayed disease response was recorded with decreasing relative humidity levels. Amongst the different levels of relative humidity, level of 100% was found optimum manifesting the initial symptom in minimum incubation period (86.4 h) and highest apparent infection rate (0.39unitday$^{-1}$) followed by relative humidity level of 95%. Minimum disease severity was observed at relative humidity level of 75 %with minimum apparent infection rate (0.28unit day$^{-1}$) and maximum incubation period (140.2 h) (Table 5).Similar studies were conducted by various workers indicating that the relative humidity of more than 90% was favourable for rapid bacterial blight development, whereas the disease ratings were relatively reduced below 75% relative humidity (Gilbertson and Maxwell, 1992; Dowson et al., 2000 and Akhavan et al., 2009).

*3.3.3. Leaf wetness*

The effect of leaf wetness period revealed that 24 h was found most suitable time periodwith minimum incubation period (88.2 h) and highest apparent rate of infection (0.37 unit day$^{-1}$) followed by leaf wetness period of 16 h and 8 h (Table 6). The pathogen failed to develop any symptoms at leaf wetness period of 2 h and 4 h.The results may be due to

Table 5: Bacterial blight progression at different relative humidity levels

| Relative Humidity (%) | Incubation period (h) | % disease severity after days of incubation period | | | | Mean | Apparent infection rate (r) (unit day$^{-1}$) |
|---|---|---|---|---|---|---|---|
| | | 2 | 4 | 6 | 8 | | |
| 75 | 140.20 | 5.20 (12.80) | 19.20 (25.91) | 31.20 (33.92) | 48.40 (44.05) | 32.93 (34.63) | 0.28 |
| 85 | 121.20 | 9.60 (17.90) | 37.20 (37.55) | 56.80 (48.90) | 77.60 (61.82) | 57.20 (49.42) | 0.33 |
| 90 | 107.40 | 11.20 (19.30) | 54.80 (47.75) | 70.80 (57.31) | 85.20 (67.50) | 70.26 (57.51) | 0.35 |
| 95 | 98.60 | 12.40 (20.40) | 56.80 (48.90) | 74.40 (59.65) | 87.60 (69.60) | 72.93 (59.38) | 0.36 |
| 100 | 86.40 | 15.20 (22.80) | 57.60 (49.35) | 78.40 (62.37) | 94.00 (75.98) | 76.66 (62.58) | 0.39 |
| Mean | | 10.70 (18.60) | 45.12 (41.90) | 62.32 (52.43) | 78.56 (63.80) | | |
| SEm± | 1.74 | Relative Humidity = 0.63, Interval = 0.57<br>RH × Interval = 1.27 | | | | | |
| CD ($p$=0.05) | 5.17 | Relative Humidity = 1.79, Interval = 1.60<br>RH × Interval = 3.59 | | | | | |

the reason that under consistently water-soaked conditions, the bacterium *Xanthomonas axonopodis*pv. *phaseoli* undergoes rapid proliferation and colonization within the intercellular fluid. The results were in consonance with the findings of Shukla and Gupta (2005) as they reported leaf wetness above 24 h as an important requisite for the development of bacterial spot of tomato. Studies conducted by Pria et al. (2006) also gave similar results revealing maximum disease severity at leaf wetness duration of 24 h due to infection of *Xanthomonas axonopodis*pv. *phaseoli* on citrus plants.

Table 6: Bacterial blight progression at different leaf wetness durations

| Leaf Wetness (h) | Incubation period (h) | % disease severity after days of incubation period | | | | Mean | Apparent infection rate (r) (unit day$^{-1}$) |
|---|---|---|---|---|---|---|---|
| | | 2 | 4 | 6 | 8 | | |
| 2 | - | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | - |
| 4 | - | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | - |
| 8 | 116.20 | 5.20 (11.20) | 12.80 (20.81) | 20.00 (26.32) | 29.60 (32.90) | 20.80 (26.68) | 0.22 |
| 16 | 97.80 | 6.40 (14.63) | 14.40 (22.07) | 24.80 (29.81) | 43.20 (41.06) | 27.46 (30.98) | 0.24 |
| 24 | 88.20 | 15.20 (22.84) | 54.80 (47.74) | 77.60 (61.82) | 91.60 (73.43) | 74.66 (60.98) | 0.37 |
| Mean | | 8.94 (16.30) | 16.40 (18.12) | 24.48 (23.60) | 32.88 (29.48) | | |
| SEm± | 1.38 | Leaf Wetness = 0.58, Interval = 0.52<br>Leaf Wetness × Interval = 1.16 | | | | | |
| CD ($p$=0.05) | 4.12 | Leaf Wetness = 1.64, Interval = 1.47<br>Leaf Wetness × Interval = 3.29 | | | | | |

(Figures in parentheses are arc sign transformed)

## 4. CONCLUSION

High temperature (30°C) coupled with high relative humidity (>90%) and leaf wetness period (>16 h) were the most conducive environmental factors responsible for the disease development.

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